Efficacy Test of Chordin Morpholinos. CBK for the UO team, 15 October 2006

Summary

We compared the quality of 5 chordin MO batches supplied by Gene Tools, designated E, F, G, H, and I, using a 'blind' experimental design. Injecting any of these yielded a specific chordin-knockdown phenotype. We did not detect significant differences in phenotype and mortality among the batches.

Objective

To learn if major differences in MO efficacy are present among separate productions.

Method

Injections: To minimize experimental noise we examined the five MOs injected on the same day and scored all the results together. Three nanoliters of each MO (5) at two concentrations (3 & 5 mg/ml; 5x2=10), were injected into sets of 50 embryos. Each MO injection was duplicated (10x2=20) with the sets done blindly and in random order by two different individuals, two being required because of the large size of the experiment. Several days before the experiment they did a preliminary run with MO batch A to determine the effective concentrations, coordinate the size of the injected bolus, and work out a design for phenotypic scoring. We used eggs obtained from spontaneous spawnings from tanks of our "injection fish" and, later in the morning, these were supplanted with batches of artificially fertilized eggs to increase numbers, hence there was quite a diversity in the batches of eggs being injected. Two non-injected sets of eggs, one from the injection fish and the other from artificial fertilization, were included as controls.

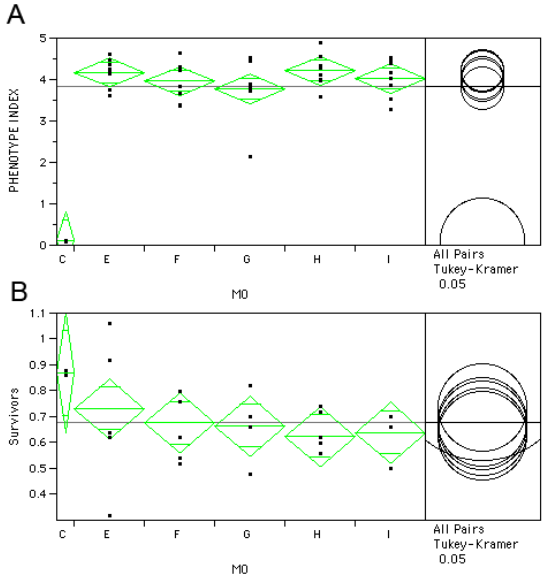

Fig. 1. Mean scores. Diamonds show 95% confidence limits (vertical) and numbers in the group (horizontal). Circles show significant differences if not overlapped (P<0.05), as for control (c) vs. injected embryos in A. The statistics use a simplifying assumption that each of the 40 samples is independent.

Scoring was done at 2 dpf, by 4 individuals (blind, batches randomly assigned); each set was scored twice (20x2=40). Three phenotypes were scored – tail fin defect, blood cell pool, and eye size. For each, one of three phenotypic ranks was assigned to each embryo – normal, mild, severe – and the totals were recorded.

Analysis. Ranks were converted to numbers – 0 (normal), 1, 2 – and a separate score for each phenotype tabulated by multiplying the number of embryos in each class by the numerical rank for that class, summing the three classes, and dividing the sum by the total number of embryos scored, yielding a score between 0 and 2 for each set. The phenotypic score is defined as the sum of the three individual scores (fin, blood, eye; maximum = 6). Excel and JMP software was used for statistical analyses.

Results

We found that injected embryos had an overall phenotypic score of 4.03, and survival of 67% whereas uninjected controls had phenotypic score of 0.01 and survival of 87%. There were no significant differences among the MOs for phenotypic score (Fig. 1A) or survival (Fig. 1B). Neither were the phenotypic scores significantly different if partitioned by MO concentration (3 and 5 mg/ml; ANOVA, means 4.01 vs. 4.06; $F_{1,39}$=0.11; P=0.75) or by who injected, or scored the embryos (not shown). Table 1 shows the averaged scores for the 5 MOs at the two concentrations, and at both concentrations averaged together ("overall").

Table 1: Phenotypic scores, partitioned by MO and concentration. For the injected embryos, none of the categories differ significantly

	Score				
Concentration	MO				
	E	F	G	H	I
None, score = 0.01					
3mg/ml	4.54	4.48	3.80	4.30	3.40
	4.30	4.26	3.01*	4.03	3.94
AV	4.42	4.37	3.41	4.17	3.67
5mg/ml	3.68	3.62	4.49	3.79	4.31
	4.18	3.51	3.81	4.73	4.46
AV	3.93	3.57	4.15	4.26	4.39
OVERALL	4.18	3.97	3.78	4.21	4.03

*Each person injecting this set noted clogging, which might account for the lower score.

Comment

We would feel comfortable doing experimental studies with any of these batches. In retrospect we might have used a wider difference between the two concentrations. However, the preliminary experiment revealed quite a low penetrance (<10%) with injection at only 1 mg/ml of the A MO, reputed to be 'good'. Hence we settled on the two higher concentrations.